

**CHROMOSOME ANALYSIS AND HETEROCHROMATIN
RECOGNITION IN THE SOUTHERN AFRICAN SPECIES OF
ORNITHOGALUM: 1. *ORNITHOGALUM SEINERI* (ENGL. & KR.) OBERM.**

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ABSTRACT

Ornithogalum seineri is an allohexaploid species with $2n = 6x = 24$. Its chromosome complement is bimodal with a basic number of $x = 4$, composed of three large (L) and one small (S) chromosomes. Quinacrine fluorescence staining shows a complex pattern of enhanced fluorescence bands only in the L-chromosomes.

UITTREKSEL

**CHROMOSOOM ANALISE EN HETEROCHROMATIEN UITKENNING IN DIE
SUIDELIKE AFRIKA SOORTE VAN *ORNITHOGALUM* 1. *ORNITHOGALUM SEINERI*
(ENGL. & KR.) OBERM.**

Ornithogalum seineri is 'n alloheksaploïede soort met $2n = 6x = 24$. Die chromosoom komplement is bimodaal met 'n basiese getal van $x = 4$, saamgestel uit die drie groot (L) en een klein (S) chromosome. Quinakriene fluoressensie toon 'n komplekse patroon van verhoogde fluoresserende bande slegs in die L-chromosome.

INTRODUCTION

The genus *Ornithogalum* in Southern Africa has been recently revised by Obermeyer (1978). It includes 54 species subdivided into three sub-genera. Only less than a third of the species have been examined cytologically.

In recent years the use of fluorochrome staining, and other advanced staining techniques for the linear differentiation of chromosomes, has revolutionised karyotype analysis and the understanding of chromosome structure (Vosa, 1975; 1977).

The present study, the first of a series, is concerned with the chromosome analysis and heterochromatin recognition of *Ornithogalum seineri* (Engl. & Kr.) Oberm., using the fluorochrome Quinacrine and Feulgen staining.

MATERIAL AND METHODS

The material consisted of plants collected in the wild by the present author in three localities in the Transvaal and subsequently cultivated in the cool greenhouses at the Botany School, Oxford. The localities of collection and the author's collecting numbers are indicated in Table 1.

For the cytological preparations generally, actively growing root-tips were pretreated in 0.05 % colchicine in distilled water at room temperature for 3-4 hours, and then fixed in 1:3 acetic alcohol overnight.

TABLE 1.
Collecting numbers and localities of collection of *Ornithogalum seineri*.

Collecting number	Localities of collection
1. <i>C. G. Vosa 445</i>	2331 (Phalaborwa): Phalaborwa (-AD).
2. <i>C. G. Vosa 1653</i>	2428 (Nylstroom): Potgietersrus (-AB).
3. <i>C. G. Vosa 1668</i>	2528 (Pretoria): Pienaarsrivier (-BB).

The Feulgen preparations were made according to the schedule suggested by Darlington and La Cour (1976) and the Quinacrine fluorescence preparations according to the schedule suggested by Vosa (1970; 1973).

RESULTS AND DISCUSSION

The Feulgen stained somatic chromosomes of *Ornithogalum seineri* are illustrated in Figure 1. The karyotype consists of 24 chromosomes and includes 18 large (L) and 6 rather small acrocentric chromosomes (S). The S-chromosomes are about one fourth of the L-chromosomes. Thus, the complement of *O. seineri* presents a striking bimodality, which is indeed found in many species of the subgenus *Urophyllon* (Vosa, in prep.).

There are up to six L-chromosomes with a nucleolar attachment distally located in the short arm, but only two are usually visible in most metaphases (Fig. 1).

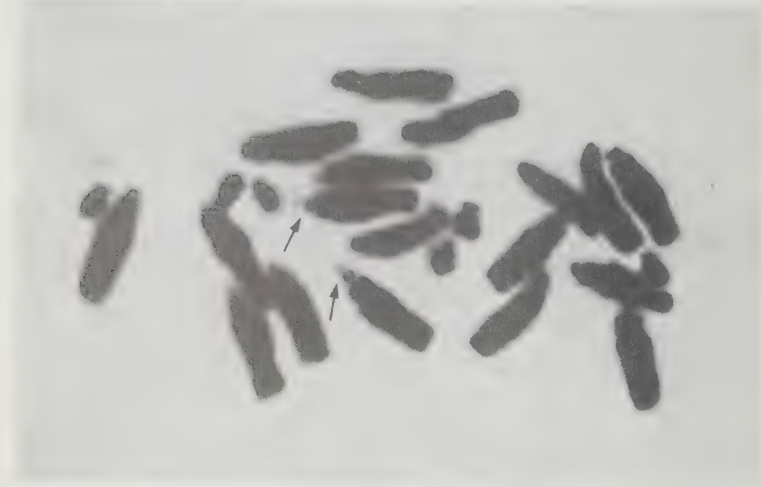


FIG. 1.

The Feulgen stained somatic chromosomes of *Ornithogalum seineri*. The arrows indicate the two most prominent nucleolar constrictions ($\times 2500$).

Figure 2 shows the chromosome complement, stained with Quinacrine and Figure 3 its diagrammatic representation.

Quinacrine staining shows at once a complex pattern of enhanced fluorescence bands (Q-bands) in all L-chromosomes and, on the basis of such patterns and size, they can be subdivided into three groups of six chromosomes. The S-chromosomes do not show any banding but, on the basis of their morphology, they can be subdivided also into three groups.

The repetitive band patterns of the L-chromosomes and the overall morphology of the S-chromosomes indicate that *O. seineri* is hexaploid with a basic number of $x = 4 (3L + 1S)$.

It is known that Quinacrine fluorescence is enhanced by the presence of repetitive sequences of Adenine-Thymidine (AT) nucleotides and that Guanine-Cytidine (GC) nucleotides effectively quench its fluorescence, even when they are regularly intercalated in an otherwise AT-rich sequence (Weisblum, 1973; Weisblum and de Haseth, 1972 and see Vosa, 1975 for discussion). The results show that the repetitive DNA sequences, which represent the heterochromatic segments of *O. seineri*, are composed, probably almost exclusively, of AT-nucleotides.



FIG. 2.

The Quinacrine stained chromosomes of *Ornithogalum seineri*. Note the despiralised procentric segments of most of the chromosomes (arrows), $\times 2500$.

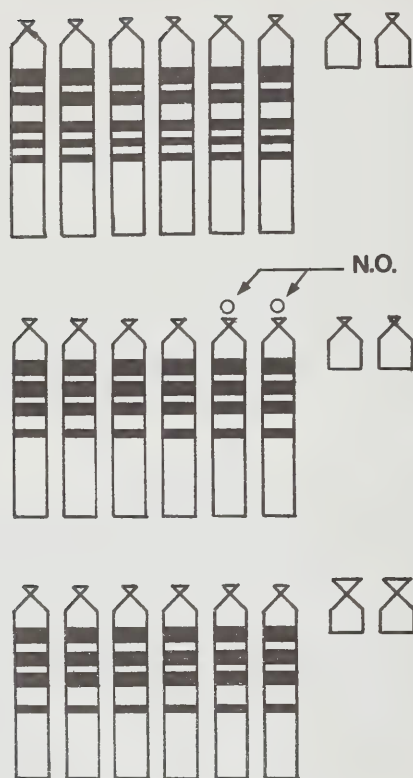


FIG. 3.

The diagrammatic representation of the Quinacrine stained karyotype of *Ornithogalum seineri*. Note the most prominent nucleolar chromosomes (N.O.).

It is interesting to note that, besides producing discrete fluorescent patterns, Quinacrine staining seems to despiralise, or otherwise stretch, the chromosome segments between the centromere and the first band in all L-chromosomes (Fig. 2, arrows). The mechanism of such an occurrence is not known at present but it resembles the *in vivo* despiralising effect of the fluorochrome Hoechst 33258 on the heterochromatic segments of some mammalian chromosomes (Hilwig and Gropp, 1973). In the case of *O. seineri*, however, the despiralisation occurs in fixed chromosomes and in non-heterochromatic segments.



FIG. 4.
Interphase nucleus of *Ornithogalum seineri*. Note the fluorescent chromocentres ($\times 2500$).

The interphase nuclei of *O. seineri* present prominent chromocentres which vary in number with a minimum of about 4–5 (Fig. 4). In middle prophase nuclei, the despiralised euchromatic segments can be seen attached to four or more highly fluorescent chromocentres (Fig. 5).

The three collections examined are very similar to one another both cytologically and morphologically.

O. seineri is a widely distributed species (Obermeyer, l.c.). In all three localities of collection the species occurs in drifts of up to one hundred plants, mostly of flowering size but with a fair number of smaller plants and seedlings, showing a high degree of fertility.

Preliminary observations of meiotic stages have shown regular bivalent formation and pollen fertility seems to be about 75–80%.

The results show that *O. seineri* is an old established allopolyploid species, well adapted to dry conditions and found, typically, in overgrazed sandy flats, where it is probably protected by its alleged poisonous properties.

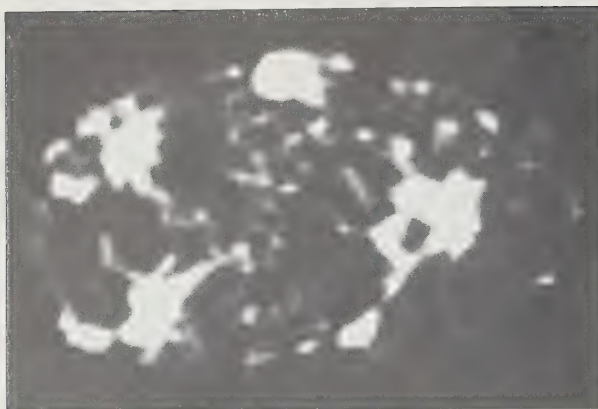


FIG. 5.

Middle prophasic nucleus of *Ornithogalum seineri*. The euchromatic chromosome segments are attached to the chromocentres ($\times 2500$).

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REFERENCES

- DARLINGTON, C. D. and LA COUR, L., 1976. *The Handling of Chromosomes*. London: G. Allen & Unwin Ltd.
- HILWIG, I. and GROPP, A., 1973. Decondensation of constitutive heterochromatin in L-cells by a bibenzimidazole compound (Hoechst 33258). *Expl Cell Res.* **81**: 474-477.
- OBERMEYER, A. A., 1978. *Ornithogalum*: a revision of the Southern African species. *Bothalia* **12**: 323-376.
- VOSA, C. G., 1970. Heterochromatin recognition with fluorochromes. *Chromosoma* (Berl.) **30**: 366-372.
- VOSA, C. G., 1973. Heterochromatin recognition and analysis of chromosome variation in *Scilla sibirica*. *Chromosoma* (Berl.) **43**: 269-278.
- VOSA, C. G., 1975. The use of Giemsa and other staining techniques in karyotype analysis. *Curr. Adv. Pl. Sci.* **6**: 495-510.
- VOSA, C. G., 1977. Heterochromatin patterns and species relationship. *Nucleus, Calcutta* **20**: 33-41.
- WEISBLUM, B., 1973. Fluorescent probes of chromosomal DNA structure: three classes of acridines. *Cold Spring Harb. Symp. quant. Biol.* **38**: 441-449.
- WEISBLUM, B. and DE HASETH, P. L., 1972. Quinacrine: a chromosome stain specific for deoxyadenylate-deoxythymidylate-rich regions of DNA. *Proc. natn. Acad. Sci. U.S.A.* **69**: 629-632.